Bacterial vaginosis: A diagnostic approach

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Introduction

Bacterial vaginosis is the commonest cause of abnormal vaginal discharge in Western countries. It is a mild condition which nevertheless can cause considerable inconvenience. There is the possibility that the organisms associated with vaginosis may be associated with preterm labour. Bacterial vaginosis is still poorly understood and there is confusion both about how it should be diagnosed and the role of the microbiology laboratory. In order to dispel some of this confusion we have reviewed the aetiology, pathogenesis and diagnosis of bacterial vaginosis and made recommendations on the diagnostic procedures appropriate for genitourinary medicine and gynaecology clinics and for general practitioners. The microbial flora of the vagina in normal women[1][2] and those with bacterial vaginosis[3] have recently been reviewed and will not be discussed in depth in this article.

History of bacterial vaginosis

Nomenclature

In 1954 Gardner and Dukes[4] described a distinct clinical entity which presented as an increased often foul smelling vaginal discharge that was not associated with any recognised pathogen. They named this condition "*Nonspecific vaginitis*" in order to distinguish it from other causes of vaginitis such as *Trichomas vaginalis* and *Candida* spp. The isolation of *Haemophilus vaginalis*, subsequently named as *Corynebacterium vaginale* and now known as *Gardnerella vaginalis*, from these patients caused Gardner and Dukes to change the name to *Haemophilus vaginalis* vaginitis.[5] This term, later modified to *Gardnerella*-associated vaginitis, was used by many workers[6][7] until it became clear that anaerobes were also present in this condition and the term anaerobic vaginitis or vaginosis[8] was favoured. In 1984 a working group reached a consensus that it would be more appropriate to call this condition "bacterial vaginosis";[9] "bacterial" because of its association with many bacteria and "vaginosis" because of the lack of an inflammatory response. Recently, vaginal bacteriosis has been suggested as a more correct name[10][11] but bacterial vaginosis remains the most widely used and accepted term.

Symptoms

Despite the controversy regarding the naming of this condition the original description by Gardner and Dukes[4][5] remains the classical and most accurate definition. There is an increased vaginal discharge and the smell, which is characteristic and often described as fishy, is most often present after menstruation or sexual intercourse. The severity of the symptoms can vary from mild to florid. However, this is essentially a mild condition and patients vary in their tolerance of the increased vaginal discharge, which may have been present for many months or years.

Signs

The signs of bacterial vaginosis as seen by the clinician include; a vaginal discharge which is homogeneous in nature and appears to adhere to the vaginal wall in a thin film and can vary from white to grey in colour; a fishy smell that can be detected by the addition of 10% sodium hydroxide to fresh vaginal discharge which converts the non-volatile salts into highly voltatile and odourous free bases; increased vaginal pH and the presence of "clue cells" and Gram variable bacilli. In order to establish a uniform approach these signs were accepted as criteria which would define the diagnosis of bacterial vaginosis (BV) in 1984.[12]

Bacterial associations

The bacteria associated with bacterial vaginosis include *G. vaginalis*,[5][13][14] *Bacteroides* spp.[13-17] particularly *B. bivius*, *B. disiens*, and the black pigmented *Bacteriodes* now known as *Porphyromonas* spp. and *Prevotella* spp., *Peptostreptococcus* spp.,[15-18] *Mycoplasma hominis*[13][19] and *Mobiluncus* spp.[20-23] All of these bacteria, with the possible exception of *Mobiluncus* spp., also colonise normal women albeit in smaller numbers.[13][24][25] *Mobiluncus* spp. have been found seldom as members of the endogenous flora.[20][22] This may be because of the difficulty in isolating and identifying such a fastidious organism. In addition to the increase in these bacteria there is a concomitant decrease in lactobacilli which usually predominate in the normal healthy vagina.

Aetiology

Evidence that there is a strong bacterial association in BV is clear. Despite the years that have passed since the first description by Gardner and Dukes[4][5] it is still unclear whether this association indicates a true pathogenic role. They believed that *G. vaginalis* was the causative agent and inoculated volunteers with pure cultures of *G. vaginalis* and vaginal-material from patients with BV.[5][26] Some of the volunteers became colonised with *G. vaginalis*, the criteria used by Gardner and Dukes for BV.

However, subsequent isolation of *G. vaginalis* from normal women and the possibility of a mixed infection with anaerobes has shed doubt on the interpretation of their findings.

Gardner and Dukes also found that 90% of male sexual partners of women with BV were colonised with *G. vaginalis*[5] suggesting that sexual transmission may occur and hence implying a causative role. Colonisation of the male urethra[27] and semen[28] has since been demonstrated but the association found by Gardner *et al*[5] with male sexual partners has proved difficult to reproduce. In addition treatment of the male partner with metronidazole has not decreased the recurrence rate of BV in women.[29-31]

The only predisposing factor for BV found consistently by different studies is aspects of sexual activity such as longer history of coital experience,[32] a greater number of sexual partners[16 33] and the presence of other sexually transmitted infections particularly *Trichomonas vaginalis*.[32] Patients using an intrauterine device for contraception[32] also appear more susceptible but this is more likely to be caused by disruption of the mucosa than by any host factors.

The aetiological agent(s) of bacterial vaginosis remain unknown. However, the condition is marked by a distinct change in vaginal ecology which results in a loss of lactobacilli, increase in other flora and a rise in vaginal pH.

Published diagnostic methods
Over the years a number of different methods have been used to diagnose bacterial vaginosis, not all of which are appropriate for routine diagnosis. These techniques, their rationale and limitations are discussed in this section.

Cultural techniques
The seminal work of Gardner and Dukes[5] in defining bacterial vaginosis, or non-specific vaginitis as it was then known, dominated the approach to its diagnosis for over twenty-five years. They described a close association between bacterial vaginosis and the isolation of *G. vaginalis* from the vaginal discharge of women with this condition. They did not isolate *G. vaginalis* from women with a normal vaginal discharge. Despite their subsequent failure to prove a pathogenic role for *G. vaginalis* by fulfilling Koch's postulates, the idea was established that vaginosis was an infection in which *G. vaginalis* was a good marker, if not actually the cause of the condition. Over the next 25 years a variety of methods was used to culture and identify *G. vaginalis* as a means of diagnosing vaginosis.

While growing *G. vaginalis* on blood or chocolate agar was straightforward, identifying it was not. Dunkelberg *et al*[34] used a peptone starch dextrose agar and an identification scheme which was both time consuming and demanding. Human blood agar, particularly in a bilayer plate,[25] largely solved the identification problem as *G. vaginalis* produced a diffuse beta haemolysis on this medium. However, with the use of human blood agar, it soon became clear

that *G. vaginalis* could be found in up to 40% of women without vaginosis.[24 25] At the same time the potential role of other organisms in vaginosis was being recognised e.g. *M. hominis*, *Mobiluncus* spp., *Bacteroides* spp. and anaerobic cocci. Culture of *G. vaginalis* was not a satisfactory diagnostic technique for vaginosis. Culture of many of the other species associated with the condition was again difficult and time consuming. Culture is important in research studies on vaginosis, but it is unnecessary and can be misleading in routine clinical work.

Non-cultural techniques
Problems with the use and interpretation of culture as a means of diagnosis led to the consideration of non-cultural methods. Spiegel *et al*[15] analysed the pattern of non-volatile fatty acids in vaginal discharge by gas liquid chromatography. Whereas in normal women the lactate was the main component present with low levels of succinate, in women with vaginosis the succinate : lactate ratio rose to ≥ 0.4. Chen *et al*[35 36] detected the diamines, putrescine and cadaverine, in vaginal washings from women by thin layer chromatography. These amines are responsible for the fishy odour in the potassium hydroxide "sniff" test.

An alternative approach is the detection of proline aminopeptidase in vaginal secretions. Elevated levels of proline aminopeptidase have been shown to predict accurately women with a clinical diagnosis of BV[37 38] and those diagnosed using the Gram stain.[38]

Such tests can be used to diagnose vaginosis but require relatively sophisticated laboratory procedures, are not rapid and move the diagnosis away from the patient. As with culture, they have their place in research on vaginosis and in clinical trials of new therapies but are not practical for general clinical use.

The other noncultural diagnostic technique is the Gram stain. The "clue cell" is a squamous epithelial cell covered with small Gram variable bacilli which is characteristic of vaginosis. However, it is not necessary to see "clue" cells to make the diagnosis. The key feature is the absence of typical large Gram positive bacilli (lactobacilli) and their replacement with Gram variable or Gram negative rods. Spiegel *et al*[39] tried to put this on a more systematic basis by a scoring system for these morphotypes. This principle has been used by Nugent *et al*[40] to develop a new scoring system, again comparing lactobacillus morphotypes with gardnerella and bacteroides morphotypes. Initial results suggest that such an approach is robust and that microbiologists can be trained to a high level of performance within a short period of time.[41] Krohn *et al*[42] showed, using the Spiegel system, that the Gram stain had a sensitivity of 62% and a specificity of 95%. The predictive value of a positive test was 76%. In terms of specificity and positive predictive value the Gram stain was better than GLC or *G. vaginalis* culture although it was less sensitive. In research use the Gram stain allows subsequent reassessment for independent verification and allows the

recognition of flora which is abnormal but does not fulfil the criteria for bacterial vaginosis.

This looks promising. However, it is important to remember that the "gold standard" for these more recent studies remains the clinical diagnostic criteria obtained by the physician. A systematic approach to the Gram stain, which is to be reliable, does involve focusing the attention of clinic or laboratory staff on a particular technique which must to some degree be at the expense of other duties. Studies set up to determine its potential reproducibility do not entirely reproduce the situation in an average clinic where vaginosis is but one of a range of problems.

Routine diagnoses

Having considered the various diagnostic procedures that have been described for bacterial vaginosis, in this section we consider in more detail those applicable to everyday practice.

Clinical aspects

Bacterial vaginosis is usually diagnosed after the exclusion of other genital infections. However, assessment of the compound criteria for the diagnosis of BV is seldom obscured by the presence of another infection. Indeed fungal hyphae and Gram negative intracellular diplococci may be more difficult to detect by microscopy in smears prepared from women who have BV than women who do not.

The commonest presenting symptom of women who have BV is a malodorous vaginal discharge, which is not associated with any itching or irritation. However, approximately 50% of women with BV presenting to genitourinary medicine clinics are asymptomatic. In those who present to such clinics with the symptom of an abnormal discharge, BV is commoner than either candidiasis or trichomoniasis. In the series of Eschenbach et al,[43] who studied 640 women attending a clinic for sexually transmitted diseases, 49% women with BV had noted vaginal malodour, compared with 20% of those without BV. Fifty percent of women reporting an increased vaginal discharge had BV compared to 37% of those not reporting an increased discharge. Overall, 65% of women with BV reported vaginal malodour or increased discharge.

The composite criteria for the diagnosis of bacterial vaginosis were described by Amsel et al in 1983[32] and evaluated in a study of 397 women attending a gynaecology clinic. The presence of at least three of four criteria were required: a vaginal pH > 4.5; a thin homogeneous vaginal discharge; a positive KOH test; the presence of clue cells on a wet mount. Whilst in many women with BV all four criteria will be fulfilled, the acceptance of only three of them allows for the impact of other factors which might obscure one of the criteria discussed below. It also implies that the distinction between normal and abnormal flora might be imprecise.

Vaginal pH

A pH of vaginal fluid > 4.5 is a sensitive indicator of BV but of low specificity. The pH might be elevated in women with normal vaginal flora following intercourse or at the time of menstruation.[32 44] Inadvertent inclusion of cervical mucus in the sample will produce an erroneously high pH value.[32] Thus, in the study of Amsel et al[22] 97% of women with BV had a pH > 4.5. Thomason et al[45] studied 310 women attending a gynaecology clinic. Ninety two percent (90/98) of the women with BV had a pH > 4.5, as did 35% (81/232) of those without BV. Eschenbach et al[43] reported that a threshold for the pH of > 4.7 gave the greatest diagnostic precision, but even then, 96.5% (300/311) women with BV had an elevated pH compared to 47% (166/350) of women without BV. In a study of 593 pregnant women, it was reported that 84% (61/73) women fulfilling the Gram stain criteria for BV had a pH > 4.5.[45]

Thin homogeneous vaginal discharge

Amsel et al[32] described the vaginal discharge of women with BV as having a thin, homogeneous appearance and a milk-like consistency. The amount could be scanty, moderate or profuse. Others have added that it is usually white and adherent to the vaginal walls.[46] The appearance of vaginal fluid may be altered by several factors including intercourse and douching. Failure to detect an abnormal discharge does not, therefore, exclude the diagnosis of BV. It is neither a sensitive nor a specific indicator of BV in most series. In pregnant women it was not independently related to BV after pH, KOH test and clue cells had been adjusted for in a multivariate analysis.[42] Eschenbach et al[43] detected abnormal discharge in 69% (184/266) women with BV and 3% (9/318) of those without, whilst Thomason et al[45] found it in 52% (51/98) women with BV and 22% (61/212) of those without. The higher specificity of the criterion in the Eschenbach series suggests that there is variation in the ability of clinicians to detect the discharge, or that it is more easily detected in women attending a clinic for sexually transmitted diseases than in women attending a gynaecology clinic.

Potassium hydroxide test

This was first described by Pheifer et al[13] who noted that a fishy odour was produced when 10% KOH was added to a sample of vaginal fluid from a woman with BV. Volatile polyamines, particularly putrescine and cadaverine are released from their salts by the addition of alkali, and contribute to the odour.[35 36] Trimethylamine, the predominant contributor to the smell of spoiling fish is also released.[47] In vitro, Mobiluncus spp. but not Gardnerella or two strains of Bacteroides spp. produced trimethylamine.[48] Some women with BV note that the vaginal malodour is worse following intercourse. This may be because semen, having a relatively high pH, releases the amines in a similar manner to KOH. However, putrescine is also present in semen so that women with a sensitive sense of smell might note such an odour without having BV, and "false positive" KOH tests can occur in women

who have had intercourse recently. The extent of observer variation in ability to detect the characteristic odour has not been examined. This criterion is sometimes evocatively called the "whiff test". The term "amine test" is also commonly used, although in the original paper of Amsel *et al*[32] the amine test referred to analysis of vaginal fluid by thin-layer chromatography to demonstrate the presence of putrescine and cadaverine.

The test is usually specific for BV but of moderate sensitivity. Seventy six percent of women with BV in the study of Amsel *et al*[32] had a positive KOH test. In the series of Eschenbach *et al*[43] 43% (134/311) women with BV, and 1% (3/350) women without BV had a positive test. In the series of Thomason *et al*[45] the corresponding figures were 84% (82/98) and 2·3% (5/212).

Clue cells
The anaerobic bacteria which are present in women with BV, particularly *Gardnerella*, adhere more strongly to vaginal epithelial cells as the pH increases. Clue cells are vaginal epithelial cells which are so coated with coccobacillary bacteria that when viewed on a wet mount the cell border is obscured.[5] The cells also have a stippled appearance and the nucleus may be obscured. The detection of clue cells is the single most sensitive and specific criterion for BV, but is operator dependent. Debris or degenerate cells can be mistaken for clue cells. Lactobacilli can adhere to epithelial cells in low numbers. Eschenbach *et al*[43] proposed that at least 20% of the epithelial cells should have the appearance of clue cells for the test to be positive. The absence of lactobacillus morphotypes on the wet mount can be a supportive finding in favour of the diagnosis of BV. Occasionally, cells are seen with adherent curved rods, resembling *Mobiluncus mulieris*. These have been termed "comma cells".

Clue cells were detected in 81% (251/311) of women attending a clinic for sexually transmitted diseases who had BV, and 6% (20/350) of women who did not have BV. When the criterion was changed to more than 20% epithelial cells having the appearance of clue cells the proportions became 78% (241/311) of women with and 5% (16/350) of women without BV.[43] In a series from a gynaecology clinic, 98% (96/98) of women with BV and 6% (12/212) of women without BV had clue cells.[45]

Recommendations
It is important to remember when deciding on a diagnostic approach for bacterial vaginosis, that other sexually transmitted diseases may be present in association with BV. Women with *T. vaginalis* usually have a profuse frothy discharge, and the vagina and cervix may be inflamed. The pH of the vaginal fluid is also raised, so that diagnostic confusion with BV can occur. If *T. vaginalis* is not recognised, but the woman is treated for BV with oral metronidazole, the treatment would be effective in most cases. However, epidemiological treatment of the partner(s) of a woman with *T. vaginalis* is advisable and screening for other

T. vaginalis is advisable and screening for other sexually transmitted infections should also be undertaken.

In a genitourinary medicine (GUM) clinic the diagnosis of BV is best made using all four composite criteria; (1) abnormal vaginal discharge, (2) raised pH $>4·5$, (3) KOH test and (4) characteristic microscopy. On the few occasions when the diagnosis of BV is still uncertain, a review of the Gram stain should also allow a final diagnosis to be made and for a fungal infection to be excluded. Treatment of asymptomatic women is not usually indicated. While GUM clinics should not require microbiology support to diagnose BV, collaboration between clinic and laboratory to facilitate training and audit for clinic staff who perform microscopy is valuable.

Those performing vaginal examinations outside of GUM clinics are unlikely to have the training to perform wet mount examinations of vaginal fluid reliably. Gynaecologists might have a suitable microscope available for use in infertility clinics. However, for general practitioners and most gynaecologists it is still simple to test the other criteria: abnormal discharge; pH; and KOH test, to confirm the diagnosis of BV if it is suspected. Laboratory confirmation, if desired, should not be sought from culture, but a smear of vaginal fluid can be prepared on a glass slide, air dried, and a Gram-stain reading requested. The swab should be rolled on the slide to give a thin uniform sample. Any practitioner performing vaginal examinations should have such equipment available already, for the preparation of cervical smears. Alternatively, a high vaginal swab could be sent with a request for a Gram stain to be prepared in the laboratory, but it is likely that this will produce a less satisfactory sample (see table).

Conclusions
Many physicians regard bacterial vaginosis as a harmless abnormality and do not recommend treatment in the absence of symptoms.[43] It should not, however, be dismissed as a mild condition of no consequence. Some women with recurrent BV experience considerable distress from their symptoms, particularly if the odour offends their sexual partner. Some women who have never had the diagnosis made, regard a fishy vaginal odour as normal and are enlightened when the condition is diagnosed and treated. There is increasing evidence that BV is associated with serious

Table Recommendations for the diagnosis of BV in different clinic settings

Diagnostic test	Clinic with microscopy facilities	Clinic without microscopy facilities
pH	Yes	Yes
Assessment of discharge	Yes	Yes
KOH test	Yes	Yes
Wet prep for Clue cells	Yes	No
Gram stain	For confirmation	
Laboratory tests	Not appropriate	Request Gram stain from HVS. Consider culture for *T.vaginalis*

pelvic infections in women following surgical procedures, and with adverse pregnancy outcome. Thus, BV has been strongly associated with the development of vaginal cuff infections following hysterectomy in two studies.[49][50] An association with pelvic inflammatory disease has also been postulated[43][51] although the great majority of women with BV do not have PID. Bacterial vaginosis and the organisms associated with it have been implicated in the aetiology of chorioamnionitis, preterm labour and delivery[52-54] and with postpartum maternal and neonatal infections.[55][56] If these complications are confirmed, considerable morbidity might be prevented by screening for and treating BV in women who are going to undergo pelvic surgery, and women who are or are planning to become pregnant.

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